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Sucrose application is ineffectual as a restoration aid in a transformed southern African lowland fynbos ecosystem

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Abstract

The addition of carbon (C) to the soil as sucrose has been suggested as a countermeasure to reduce plant available nitrogen (N) and increase the competitive advantage of slower growing native perennial species over faster growing annual species. To make this approach a successful restoration tool, C addition must induce the resident soil bacteria and fungi to immobilize plant available soil nutrients. In this study, both the efficacy of sucrose applications as a restoration aid and their dependence on soil microbial activity were examined in field and greenhouse trials. Carbon as sucrose (200 g m^{-2}) was added to normal and sterilized soils containing various combinations of native perennial and annual species. Their effects on soil N levels, as well as on the photosynthetic efficiency, growth and N uptake of the introduced native species, were measured. Diminished foliar chlorophyll contents, effective quantum yields ($\Delta F/Fm'$) of Photosystem II (PSII) and dry mass accumulation in response to sucrose applications were observed in both the annual and perennial introduced species, but were not reflected in corresponding reductions in soil N levels. These sucrose-induced inhibitory effects, as well as diminished plant N uptake, were more pronounced in normal than sterilized soils. This implied a bacterial component immobilizing soil N essential for plant photosynthesis and growth. However, this premise was partly contradicted by the unaltered total bacterial numbers following sucrose application in the normal soils, although coliform numbers did increase with sucrose application in these soils. These findings point to a likely abiotic mechanism of sucrose-induced inhibition of photosynthesis and growth in introduced native plants, which renders sucrose application ineffectual as a restoration aid in transformed lowland fynbos ecosystems.

Keywords: Carbon; Fynbos; Nitrogen; Renosterveld; Restoration; Sucrose

1. Introduction

Southern African Mediterranean climate fynbos ecosystems, included among 34 global biodiversity hot spots (Mittermeier et al., 2004), are heavily fragmented, with up to 95% of some, e.g. renosterveld, transformed through agriculture and viticulture (Milton, 2004). Alien grasses of temperate and subtropical origin proliferate in these highly fragmented ecosystems (Steinschen et al., 1996) and are known to impact on ecosystem structure, function and resources (D'Antonio and Vitousek, 1992). Their recent increase in abundance in lowland areas (Steinschen et al., 1996) has been attributed to habitat deterioration caused by plowing, vegetation clearing and burning, soil nutrient enrichment from surrounding agricultural areas and grazing by herbivores, which disperse the grass seeds on their hides and in their dung (Milton, 2004). This has resulted in the displacement of wildflowers that form the basis of a growing, lucrative nature-based tourist industry (Goldblatt and Manning, 2000).

Various methods for restoring transformed lowland fynbos ecosystems (especially renosterveld) invaded by alien grasses have been examined. These include removal of alien grasses by indigenous herbivores (Midoko Iponga et al., 2005;

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Shiponeni and Milton, 2006), mowing, hand clearing, light and intense burning and pre-emergent herbicide application (Musil et al., 2005). An empirical appraisal of the cost effectiveness of different labor-intensive strategies for alien grass removal, linked to a national poverty relief program, concluded that effective long-term control of the invasive grasses required an integrated management approach that would seek to limit factors that promoted their success, such as soil N enrichment (Musil et al., 2005). However, a subsequent study that examined the effects of soil nutrient depletion by carbon-rich mulching on alien grass cover and shrub establishment in a transformed lowland fynbos community reported inconclusive effects of the mulching on soil N levels and the re-establishment of native plants (Holmes, 2008).

Soil nutrient enrichment has been shown to facilitate the competitive advantage of fast growing annuals and alien grass species over slow growing native perennial species (Eschen et al., 2006; Redente et al., 1992), which persists as long as soil nutrient levels remain high. The elevated soil nutrient levels not only impede the growth of the slow growing native species but also their establishment from seed dispersed both naturally and artificially in restoration initiatives (Kindscher and Tieszen, 1998). Different techniques have been proposed for reducing levels of growth-limiting nutrients in transformed ecosystems. These include topsoil removal, the application of Ca-, Al- and Fe-based compounds, which reduce levels of plant available P in soils (Busman et al., 2002), and organic matter additions (Eschen et al., 2006), which comprise a rich source of carbon that stimulates soil microbial activity leading to a depletion of plant available soil N (Eschen et al., 2006; Reever Morgan and Seastedt, 1999). Various sources of organic matter that are high in carbon and low in nitrogen, such as sucrose, sawdust, straw, grain hulls and chopped wood (Blumenthal et al., 2003), have been applied for decreasing the availability of essential nutrients, especially nitrogen, to plants (Blumenthal et al., 2003; Corbin and D'Antonio, 2004; Eschen et al., 2006). For this to work, the above-mentioned sources of organic matter must increase microbial N immobilization and decrease plant available N (Corbin and D'Antonio, 2004). Under decreased plant available N conditions, the growth of all vegetation would be expected to decrease, but if faster growing species are disproportionately affected by decreased soil N concentrations, slower growing native species may benefit indirectly due to reduced competition from fast growing exotic species (Corbin and D'Antonio, 2004; Eschen et al., 2006).

Previous experiments have shown that the application of various sources of organic matter lead to diminished rates of net N mineralization (Gilliam et al., 2005; Hopkins, 1998) and nitrification (Gilliam et al., 2005), and reduced ammonium (Hopkins, 1998) and nitrate (Blumenthal et al., 2003) concentrations in soils. Such organic matter additions have been applied successfully in restoration initiatives that have sought to stimulate the growth of native and late seral species in alien-invaded ex-arable lands enriched from past fertilization (Blumenthal et al., 2003; Corbin and D'Antonio, 2004).

Conditions for the decomposition of organic matter in Mediterranean climate ecosystems are poor, since microbiological activity is limited by low temperatures during the wet season, and soil moisture limits decomposers during the summer drought. This is apparent from the low decomposition rates reported in Mediterranean climate sclerophyllous vegetation relative to tropical and temperate forest, savanna and grassland vegetation types (Read and Mitchell, 1983). As a consequence, the application of rapidly decomposable sources of carbon such as industrial sucrose may provide a more efficient means of reducing available soil N than slower decomposable sources of carbon such as sawdust, reeds and chopped wood in fynbos restoration initiatives. However, a potential disadvantage associated with direct sucrose application is the reported inhibition of high exogenous sucrose concentrations on plant photosynthesis and growth (Mosaleeyanon et al., 2004), especially under photosynthetic sink limitations of high photosynthetic photon flux density (Van Quy et al., 2001). Consequently, this study examined the efficacy of sucrose applications as a restoration aid in a transformed fynbos ecosystem and its dependence on soil microbial activity.

2. Methods and materials

2.1. Study area and site

The study area was the Elandsberg Private Nature Reserve (EPNR), situated on Bartholomew's Klip farm near Hermon, approximately 25 km north of Wellington in the Cape Floristic Region of South Africa (Midoko Iponga et al., 2005). The reserve was proclaimed in 1973 (Parker, 1982) and declared a natural heritage site in 1988 (Midoko Iponga et al., 2005). The study site comprised an area of degraded natural vegetation (old field) situated in a transition zone (33°44″67′ S to 33°44″72′ S; 19°03″13′ E to 19°03″17′ E) between Swartland Shale renosterveld and Swartland Alluvium fynbos vegetation units, as defined by Mucina and Rutherford (2006). It was transformed for the cultivation of oats between 1960 and 1965, and then used for the cultivation of European pasture grasses for livestock grazing between 1965 and 1987 (Midoko Iponga et al., 2005).

Renosterveld is broadly categorized as evergreen fire-prone vegetation lacking the typical fynbos families Proteaceae and Ericaceae, dominated by small leafed asteraceous shrubs, especially *Dicerothamnus rhinocerotis* (L.f) (=*Elytropappus rhinocerotis*), popularly known as renosterbos or rhinoceros bush, with an understory of Poacaeae (grasses) and geophytes (Mucina and Rutherford, 2006). Renosterveld is ecotonal to fynbos and succulent karoo, occurring on moderately fertile, clay-rich (shale and granite derived) soils on lower mountain slopes, interior valleys and coastal forelands at annual precipitation levels of between 300 and 600 mm (Cowling et al., 1986; Mucina and Rutherford, 2006). Fynbos develops under this amount of rainfall on oligotrophic soils, and succulent karoo replaces renosterveld on fertile soils under drier conditions. Post-colonial firewood collection, burning and grazing of vegetation are thought to have shaped modern renosterveld by transforming a woody shrubland perennial grassland mosaic into a more uniform shrubland dominated by *D. rhinocerotis*,

other pyrophillic shrubs and, more recently, by invasive alien annual grasses (Cowling et al., 1986).

The dominant species in the old field at the study site comprised the Eurasian perennial grass *Cynadon dactylon* (L) (Harlan, 1970; Midoko Iponga et al., 2005), the winter growing Eurasian annual grasses *Briza maxima*, *Bromus pectinatus* and *Paspalum dilatatum* (Midoko Iponga et al., 2005; Shiponeni and Milton, 2006), and some renosterveld remnants, namely the renosterveld shrub *D. rhinocerotis* and the geophyte *Oxalis purpurea*.

2.2. Field study

The split plot experimental design comprised twelve 1.5 m main plots (blocks), spaced 1.5 m apart. These were randomized at the study site in an old field with a uniform slope, substrate and vegetation composition. The plots were each subdivided into six 0.375 m square subplots. There were two treatments, a control treatment and a soil N amendment treatment in which carbon as sucrose was added to the soil at a concentration of 200 g sucrose m^{-2}. The two treatments, each replicated six times, were randomly assigned to the main plots. Sucrose additions were performed monthly over a four-month rainy season, extending from late autumn (May 2007) to late winter (August 2007).

There were six target species, namely the typical renosterveld perennials *Olea europea* L. subsp *africana* (Mill) P. S. Green, *Salvia africana lutea* L., *Rhus lucida* L. forma *lucida*, and *Pelargonium cordifolium* (cav.) Curtis, and the alluvium fynbos perennials *Leucadandron xanthoconus* (Kuntze) K. Schum and *Leucospermum praecox* Rourke. Juveniles of these six species of similar age and size were obtained from the Kirstenbosch nursery and were randomly assigned to the six subplots in each main plot in late autumn. The procedure involved the removal of a small diameter soil core with an auger at the center of each subplot into which the juvenile plant was introduced.

2.3. Greenhouse studies

Two separate greenhouse studies were conducted, both comprising split plot experimental designs. In the first greenhouse study, forty-eight soil cores (28 cm wide × 30 cm long × 10 cm deep) were excavated from the peripheries of the main plots at the field site and placed into plastic containers of the same dimensions. They were grouped into six blocks, four of which were located on the four sides and two in the center of a passively ventilated greenhouse. The eight soil-bearing containers present in each block were arranged into four columns and two rows. The control and sucrose treatments were randomly assigned to the two rows in each block. Sucrose was applied at the same frequencies and concentrations on an area basis as in the field trial. There were four target species, namely the native annuals *Arctotis acaulis* L., *Dimorphotheca pluvialis* (L.) Moench and *Ursinia anthemoides* (L) Poir. subsp *anthemoides* and the native perennial *Rhus laevigata* L. var *villosa* (L.F) R. Fern. These four species were randomly assigned to the four columns in each block in late autumn of 2007. The procedure involved the sowing of 10 seeds of each species to depths of 25 mm, with seedlings of each species thinned to four individuals per container three weeks after germination. A fifth target species comprised the naturally occurring geophyte *O. purpurea*, the bulbs of which were a common component of the soil in all the containers. Individuals of this species were also reduced to four per container three weeks after germination and all pots were weeded weekly to remove superfluous plants.

In the second greenhouse study, an additional forty-eight soil cores excavated from the peripheries of the main plots at the field site were also arranged into four columns and two rows within each of six blocks located at the six different positions in the greenhouse. There were two soil N amendment treatments combined with two soil sterilization treatments. The soil N amendment treatments comprised a control and a sucrose addition applied at the same frequency and concentration on an area basis as in the field trial. These two nutrient amendment treatments were randomly assigned to the two rows in each block. The soil sterilization treatments comprised a control in which the soil was sieved though a 2 mm mesh to remove plant detritus and larger plant propagules, e.g. *O. purpurea* bulbs, and a heating treatment in which the soil was sieved and then exposed to 220 °C for 72 h in a forced draft oven to destroy all soil micro fauna and flora. These two sterilization treatments were randomly assigned to the four columns in each block. Two native annuals, *D. pluvialis* and *U. anthemoides*, were each randomly assigned to four of the eight soil N amendment × soil sterilization treatment combinations in each block. The same procedure in seed sowing and seedling thinning was followed as described in the first greenhouse study.

2.4. Field and greenhouse environment

Rainfall amounts were monitored hourly at the field site with a tipping bucket gage, and air temperatures were monitored hourly in the greenhouse with a thermocouple sensor, each interfaced with miniature data loggers installed in radiation shields (Watch Dog 450, Spectrum Technologies Inc., Plainfield, Illinois, USA). Air temperatures in the passively ventilated greenhouse closely approximated those outdoors. The amounts of precipitation supplied daily to the soil-bearing containers by an automated irrigation sprinkler system (*ca* 4 mm d^{-1}) approximated the daily average amount of rainfall (*ca* 5 mm d^{-1}) recorded at the study site during the winter rainy season.

2.5. Plant performance

Photosynthetic pigment and chlorophyll fluorescence measurements were conducted on the fully expanded apical leaves of all introduced species in the field and greenhouse studies during their active growing period in early spring (September), and confined to clear sky conditions between 1100 and 1300 SAST (solar noon). Foliar chlorophyll contents were measured with a chlorophyll meter (Model CCM 200, Opti Sciences Inc., Hudson, NH, USA). The chlorophyll meter uses differential transmissions at two wavelengths, infrared at 940 nm and red at 660 nm, to determine the absorbance

of chlorophyll pigments in a consistent leaf area of 0.71 cm^2. Beam energy is sampled pre- and post-transmission. The ratio of the red absorbance beam to the standardized infrared reference beam gives the chlorophyll content index, abbreviated as CCI (Cate and Perkins, 2003). Effective quantum yields (ΔF/Fm′) of Photosystem II (PSII) were measured with a modulated chlorophyll fluorometer (Model OSI FI, Opti Sciences Inc., Hudson, NH, USA) following a 0.8 s saturating light pulse of 15 000 μmol m^{-2} s^{-1}. ΔF/Fm′ was calculated as $(F_{ms}-F_s)/F_{ms}$, where F_s and F_{ms} are the initial and maximum fluorescences at a steady state.

Below- and above-ground parts of the introduced annual species in the two greenhouse studies were harvested at reproductive maturity in late spring (November 2007), and those of the introduced perennial species in the field study were harvested at the end of the dry summer season (March 2008). Plant parts were dried in a forced draft oven at 65 °C to a constant mass and weighed. Nitrogen concentrations in dry plant tissue subsamples were determined with an elemental analyer (FP 528, LECO Corporation, USA) at a combustion temperature of 900 °C (Horneck and Miller, 1998). Total N uptake by the plants was computed from the product of their total dry mass (roots plus shoots) and their measured tissue N concentrations (% dry mass/100).

2.6. Soil microbial, N and C concentrations

Soils samples of 200 cm^3 were collected from the field plots and greenhouse soil-bearing containers immediately after the target species had been harvested. Total numbers of bacterial cells present in 10 g subsamples of fresh soil diluted 10-fold were assayed applying the membrane plate streak method. The numbers of coliforms present in the diluted soil subsamples were assayed by applying the membrane filter method (Finney et al., 2003). For N and C analysis, the soil samples were dried at 30 °C in a forced draft oven, sieved to ≤2 mm particle size, and 5.0 g±0.05 g samples were placed into extraction bottles. Total soil N was analyzed by complete combustion using a Eurovector Euro EA Elemental Analyser, and labile C (readily oxidized C) was analyzed using a modified Walkley Black method as described by Chan et al. (2001).

2.7. Statistical analyses

All measurements were $\log_e$ transformed before statistical analysis to reduce the inequality of variance in the raw data. The experimental designs were not fully balanced due to dissimilar numbers of treatment replications. Consequently, an REML (residual maximum likelihood) variance component analysis (linear mixed model) was applied to test for differences in measured soil and plant species parameters between treatments using the Wald X^2 statistic generated by the REML (GENSTAT Discovery Edition 3, VSL Ltd, UK). In the field and first greenhouse study, the sucrose treatment was fitted in the fixed model and the plot (block) and treatment replications were fitted in the random model. In the second greenhouse study, the soil sterilization and sucrose treatments were fitted in the fixed model and the block and two treatment replications were fitted in the random model. Differences exceeding twice the average standard errors of differences were used to separate significantly different treatment means at $P \leq 0.05$. This is based on the fact that, for a normal distribution from REML estimates, the 5% two-sided critical value is two.

3. Results

3.1. Effects of sucrose addition on plant performance

In the field study, sucrose addition significantly ($P \leq 0.05$) decreased foliar chlorophyll in all perennial species, and also significantly ($P \leq 0.05$) reduced ΔF/Fm′ in all perennial species except *P. cordifolium* (Table 1). Similarly, in the first greenhouse study, sucrose addition significantly ($P \leq 0.05$) decreased foliar chlorophyll and ΔF/Fm′ in all three annuals and in the geophyte *O. purpurea* (Table 2). The observed reductions in foliar chlorophyll and ΔF/Fm′ in the first greenhouse study were reflected in significantly ($P \leq 0.05$) diminished dry mass in the annuals *A. acaulis*, *D. pluvialis* and *U. anthemoides*, and the geophyte *O. purpurea* (Table 2). In contrast, the observed absence of any significant ($P \geq 0.05$) reduction in biomass accumulation with sucrose addition among the introduced perennial species in the field study was a consequence of their high premature mortalities over the dry summer season (Table 2).

3.2. Effects of sucrose addition on soil N and C concentrations

In both the field study and the first greenhouse study, sucrose addition had no significant ($P \geq 0.05$) effect on soil N and C concentrations (Table 3).

3.3. Interaction between soil sterilization and sucrose addition on plant performance

In the second greenhouse study, soil sterilization significantly ($P \leq 0.05$) increased ΔF/Fm′, N uptake and plant dry mass in both the introduced annuals, *D. pluvialis* and *U. anthemoides*, and also in elevated foliar chlorophyll, although it was significant ($P \leq 0.05$) only in *D. pluvialis* (Table 4). In contrast, sucrose addition significantly ($P \leq 0.05$) decreased foliar chlorophyll, ΔF/Fm′, N uptake and plant dry mass in both the introduced annuals (Table 4). Also, there were significant ($P \leq 0.05$) two-way interactions between soil sterilization and sucrose addition on ΔF/Fm′, N uptake and plant dry mass in both annuals, which displayed proportionately greater reductions in these plant performance parameters in response to sucrose addition in normal than in sterilized soils (Table 4).

3.4. Interaction between soil sterilization and sucrose addition on soil microbial and N concentrations

In the second greenhouse study, soil sterilization significantly ($P \leq 0.05$) increased the numbers of total bacteria in soils in which the introduced annuals *D. pluvialis* and *U. anthemoides*

Table 1
Effects of sucrose additions on REML computed mean foliar chlorophyll indices, effective quantum yields (ΔF/Fm′) and dry masses ($\log_e$) of introduced native species in the field study. Treatment means in bold type with different letters significantly different (differences ≥ twice average se of differences) from controls at *P ≤ 0.05, **P ≤ 0.001.

Parameter	Treatment	*L. praecox*	*L. xanthaconus*	*O. africana*	*P. cordatum*	*R. lucida*	*S. africana*
Chlorophyll index	Control	3.041a	1.117a	0.921a	1.133a	3.185a	1.816a
	Sucrose	**1.948b**	**0.403b**	**0.267b**	**0.289b**	**1.601b**	**0.519b**
	Wald statistic	$X^2_{1,33}$=45.8**	$X^2_{1,30}$=21.0**	$X^2_{1,36}$=30.3**	$X^2_{1,24}$=11.4**	$X^2_{1,36}$=29.3**	$X^2_{1,33}$=40.4**
ΔF/Fm′	Control	−0.454a	−0.417a	−0.647a	−0.496a	−0.706a	−0.530a
	Sucrose	**−1.426b**	**−0.623a**	**−1.357b**	−0.493a	**−1.101b**	**−0.880b**
	Wald statistic	$X^2_{1,33}$=29.0**	$X^2_{1,30}$=18.4**	$X^2_{1,36}$=11.8**	$X^2_{1,23}$=0.0	$X^2_{1,36}$=10.9**	$X^2_{1,33}$=7.9*
Plant dry mass	Control	1.087a	1.477a	1.593a	0.189a	2.221a	0.632a
	Sucrose	0.814a	0.489a	1.589a	0.226a	2.323a	**2.390b**
	Wald statistic	$X^2_{1,10}$=1.2	$X^2_{1,9}$=0.8	$X^2_{1,11}$=0.0	$X^2_{1,15}$=0.1	$X^2_{1,10}$=0.8	$X^2_{1,15}$=214.1**

were cultivated (Table 5), and also reduced the N content of the soils in which these two introduced annuals were cultivated, although this was only significant (P ≤ 0.05) in *U. anthemoides*. Similarly, soil sterilization increased the numbers of coliforms in soils in which the two introduced annuals were cultivated, although only significant (P ≤ 0.05) in *D. pluvialis*. However, there were significant (P ≤ 0.05) two-way interactions between soil sterilization and sucrose addition on coliform numbers, which displayed significantly (P ≤ 0.05) greater increases with sucrose addition in normal than sterilized soils in which both *D. pluvialis* and *U. anthemoides* were cultivated (Table 5).

4. Discussion

All three annuals and the geophyte displayed diminished foliar chlorophyll, ΔF/Fm′ and dry mass in response to sucrose addition, as reported in other early seral species (Redente et al., 1992). The reductions in foliar chlorophyll and ΔF/Fm′ were also commonly observed among the introduced native perennials, although not evident in their dry mass accumulation due to their high premature mortalities (> 90%) over the dry summer season. Taken together, these findings do not conform to previous reports that sucrose applications inhibit the growth of faster growing early seral species (annuals) with high nutrient demands to a greater extent than that of slower growing late seral species (perennials) adapted to low nutrient environments (Eschen et al., 2006). The findings concur with those of Corbin and D'Antonio's (2004) that carbon additions provide no significant benefit in restoring native species diversity and biomass over the long term. However, they contrast with several other studies that reported that carbon additions, either as sucrose, sawdust or a mixture of sucrose and sawdust, stimulate the growth of native species (Blumenthal et al., 2003; Eschen et al., 2006; Reever Morgan and Seastedt, 1999) by diminishing available soil N (Blumenthal et al., 2003; Reever Morgan and Seastedt, 1999) through bacterial immobilization (Blumenthal et al., 2003; Eschen et al., 2006). However, the efficacy of these additions depend on initial soil fertility, and the quantity and form of carbon (Blumenthal et al., 2003). Indeed, sucrose additions in this study did not result in depleted soil N levels. This possibly is due to the highly acid soils (pH range: 4.7–5.6), which may have restricted bacterial soil N immobilization processes as these are most efficient in slightly acidic to neutral soils in the pH range 5.5 to 7.0 (Busman et al., 2002).

Soil sterilization, either by application of chemicals, steam or dry heat, enhances soil nutrient availability, except in N-fixing plants (Rodríguez-Echeverri and Pérez-Fernández, 2005), improves plant growth and reduces seedling mortality (Ingestad and Nilsson, 1964). An improvement in plant photosynthetic efficiency and growth in sterilized soils was also clearly evident in this study and was attributed to the release of plants from natural pathogens in compliance with the enemy release hypothesis (Mitchell and Power, 2003). Nematodes, such as *Pratylenchus* and *Paratrichodorus* spp, have been implicated in plant growth inhibition through damage to plant root systems (Zahid et al., 2002). Also noteworthy in this study was that sucrose applications resulted in much greater reductions in plant dry mass and N uptake in normal than in sterilized soils,

Table 2
Effects of sucrose additions on REML computed mean foliar chlorophyll indices, effective quantum yields (ΔF/Fm′) and dry masses ($\log_e$) of introduced native species in the first greenhouse study. Treatment means in bold type with different letters significantly different from controls (differences ≥ twice average se of differences) at *P ≤ 0.05, **P ≤ 0.001.

Parameter	Treatment	*A. acaulis*	*D. pluvialis*	*O. purpurea*	*R. laevigata*	*U. anthemoides*
Chlorophyll index	Control	1.970a	1.533a	2.266a	–	0.273a
	Sucrose	**0.345b**	**0.448b**	**1.029b**	–	**0.130b**
	Wald statistic	$X^2_{1,37}$=182.5**	$X^2_{1,41}$=34.9**	$X^2_{1,37}$=35.5**	–	$X^2_{1,35}$=6.6*
ΔF/Fm′	Control	−0.388a	−0.475a	−0.506a	−0.718a	−0.549a
	Sucrose	**−0.690b**	**−0.692b**	**−0.762b**	−0.631a	**−0.798b**
	Wald statistic	$X^2_{1,34}$=53.4**	$X^2_{1,36}$=12.1**	$X^2_{1,36}$=28.8**	$X^2_{1,26}$=2.4	$X^2_{1,36}$=6.1*
Plant dry mass	Control	−2.462a	−1.523a	0.819a	−2.912a	−1.894a
	Sucrose	**−4.283b**	**−4.135b**	**0.187b**	−3.540a	**−3.760b**
	Wald statistic	$X^2_{1,11}$=24.5**	$X^2_{1,12}$=38.4**	$X^2_{1,12}$=20.3**	$X^2_{1,45}$=2.2	$X^2_{1,12}$=10.3**

Table 3
Effects of sucrose additions on REML predicted mean soil nitrogen and carbon concentrations (log_e) in the field and first greenhouse study. Treatment means in bold type with different letters significantly different (differences ≥ twice average se of differences) from controls at *P ≤ 0.05, **P ≤ 0.001.

Treatment	Field study		Greenhouse study 1	
	Nitrogen	Carbon	Nitrogen	Carbon
Control	3.582a	4.087a	4.114a	4.231a
Sucrose	3.655a	4.068a	4.064a	4.274a
Wald statistic	$X^2_{1,12}$=0.5	$X^2_{1,12}$=0.1	$X^2_{1,12}$=0.4	$X^2_{1,12}$=0.1

which implies a bacterial component immobilizing the soil N essential for plant photosynthesis and growth (Field and Mooney, 1986). Indeed, interactions between soil microbial communities may be altered by sucrose addition (Alexander, 1977), leading to a proliferation of sucrose-utilizing microbial species (Shaban, 1996) and an increased production of metabolites of fermentation, such as acetate, which is typical of sucrose-amended soil (Paul et al., 1989) and may retard root elongation and inhibit plant growth (Blank and Young, 2009). However, there were contradictions in this study's findings. These included the smaller numbers of total bacteria as well as numbers of coliforms measured in normal than in sterilized soils. This is a possible consequence of reduced competition and predation from other soil micro flora and fauna that were exterminated during soil sterilization. Also, the observed absence of any increase in total bacterial numbers following sucrose application in normal soils, with the exception of coliforms, generally concurred with similar reports in other studies (Blumenthal et al., 2003; Eschen et al., 2006).

The above-mentioned contradictions point rather to an abiotic mechanism of sucrose-induced inhibition of plant photosynthesis and growth. Several studies have reported that exogenous sucrose additions reduce foliar concentrations of both chlorophyll *a* and *b* and net photosynthetic rates (Mosaleeyanon et al., 2004). This is attributed to the accumulation of hexose (Hdider and Desjardins, 1994) and starch in chloroplasts (Mosaleeyanon et al., 2004), causing feedback inhibition of photosynthesis and consequent decreased plant growth (Hdider and Desjardins, 1994). The addition of exogenous sucrose at high concentrations has also been shown to inhibit both root and shoot growth in rice, hypocotyl elongation (Jang et al., 1997) and light-induced cotyledon opening (Dijkwel et al. 1997; Jang et al. 1997) of *Arabidopsis thaliana* seedlings (Ohto et al., 2001). It also causes flowering delays in *A. thaliana*, the latter attributed to a metabolic rather than an osmotic effect (Ohto et al., 2001).

In view of this study's findings, the application of carbon as sucrose to soils in transformed fynbos ecosystems is discouraged, as it does not improve the competitiveness of indigenous over alien species due to its cosmopolitan inhibition of plant photosynthesis and growth. Even though sucrose addition has been shown to be beneficial in restoring other ecosystems, such as prairie (Blumenthal et al., 2003) and short grass steppe (Paschke et al., 2000), its application is expensive relative to other carbon sources and therefore is less practical as a restoration tool (Reever Morgan and Seastedt, 1999). Other sources of carbon, such as sawdust, reeds, chopped wood or a mixture of

Table 4
Effects of sterilization and sucrose addition on REML computed mean foliar chlorophyll indices, effective quantum yields (ΔF/Fm′), N uptake and dry masses (log_e) of *D. pluvialis* and *U. anthemoides* in the second greenhouse study. Treatment means in bold type with different letters significantly different from controls (differences ≥ twice average se of differences) at *P ≤ 0.05, **P ≤ 0.001.

Treatments		*Dimorphotheca pluvialis*				*Ursinia anthemoides*			
1	2	Chlorophyll index	ΔF/Fm′	N uptake	Dry mass	Chlorophyll index	ΔF/Fm′	N uptake	Dry mass
Normal soil		1.909a	−0.4989a	2.052a	−0.846a	0.1573a	−0.5385	1.330a	−2.210a
Sterilized soil		**2.899b**	**−0.3185b**	**4.650b**	**1.586b**	0.3166a	**−0.2946b**	**4.332b**	**2.407b**
	Control	3.087a	−0.2388a	3.966a	1.199a	0.3538a	−0.2645a	3.703a	1.343a
	Sucrose	**1.721b**	**−0.5787b**	**2.736b**	**−0.459b**	**0.1201b**	**−0.5686b**	**1.959b**	**−1.147b**
Normal soil	Control	2.571a	−0.2432a	2.974a	0.479a	0.296a	−0.2652a	2.511a	−0.322a
	Sucrose	**1.247b**	**−0.7547b**	**1.130b**	**−2.172b**	**0.187b**	**−0.8119b**	**0.148b**	**−4.098b**
Sterilized soil	Control	3.603a	−0.2344a	4.958a	1.919a	0.412a	−0.2638a	4.895a	3.008a
	Sucrose	**2.195b**	−0.4026a	4.341a	1.254a	**0.222b**	−0.3253a	3.770a	**1.805b**
Wald statistics									
Main effects									
Sterilization		$X^2_{1,288}$=18.9**	$X^2_{1,290}$=6.2*	$X^2_{1,39}$=99.7**	$X^2_{1,48}$=70.2**	$X^2_{1,252}$=2.4	$X^2_{1,252}$=5.2*	$X^2_{1,48}$=277.7**	$X^2_{1,48}$=453.3**
Sucrose		$X^2_{1,288}$=81.6**	$X^2_{1,290}$=20.3**	$X^2_{1,39}$=16.7**	$X^2_{1,48}$=16.9**	$X^2_{1,252}$=45.0**	$X^2_{1,252}$=9.9*	$X^2_{1,48}$=57.8**	$X^2_{1,48}$=76.2**
Two-way interactions									
Sterilization × sucrose		$X^2_{1,288}$=0.1	$X^2_{1,290}$=5.6*	$X^2_{1,39}$=4.5**	$X^2_{1,48}$=6.1*	$X^2_{1,252}$=1.5	$X^2_{1,252}$=7.2*	$X^2_{1,48}$=7.3*	$X^2_{1,48}$=20.3**

Table 5
Effects of sterilization and sucrose addition on REML computed mean soil N concentrations, total bacterial and coliform numbers ($\log_e$) of soils in which *D. pluvialis* and *U. anthemoides* were cultivated in the second greenhouse study. Treatment means in bold type with different letters significantly different from controls (differences ≥ twice average se of differences) at *P ≤ 0.05, **P ≤ 0.001.

Treatments		*Dimorphotheca pluvialis*			*Ursinia anthemoides*		
1	2	Soil N concentration	Soil bacterial numbers	Soil coliform numbers	Soil N concentration	Soil bacterial numbers	Soil coliform numbers
Normal soil		4.219a	15.12a	7.269a	4.331a	15.14a	8.130a
Sterilized soil		4.095a	**16.60b**	**8.187b**	**4.120b**	**16.84b**	8.049a
	Control	4.089a	15.59a	7.317a	4.225a	15.90a	8.036a
	Sucrose	4.225a	16.13a	**8.138b**	4.225a	16.08a	8.143a
Normal soil	Control	4.082a	14.71a	6.554a	4.309a	15.11a	7.792a
	Sucrose	4.356a	15.53a	**7.983b**	**4.352b**	15.18a	**8.305b**
Sterilized soil	Control	4.097a	16.46a	8.079a	4.141a	16.69a	8.280a
	Sucrose	4.093a	16.73a	8.294a	**4.099b**	16.99a	7.981a
Wald statistics							
Main effects							
Sterilization		$X^2_{1,48}=2.9$	$X^2_{1,24}=9.1$*	$X^2_{1,24}=6.4$*	$X^2_{1,48}=6.4$*	$X^2_{1,24}=49.0$**	$X^2_{1,23}=1.0$
Sucrose		$X^2_{1,48}=2.0$	$X^2_{1,24}=1.7$	$X^2_{1,24}=11.0$**	$X^2_{1,48}=0.0$	$X^2_{1,24}=0.4$	$X^2_{1,23}=0.5$
Two-way interactions							
Sterilization × sucrose		$X^2_{1,48}=2.1$	$X^2_{1,24}=0.4$	$X^2_{1,24}=6.0$*	$X^2_{1,48}=0.2$	$X^2_{1,24}=0.1$	$X^2_{1,23}=7.1$*

these, which have been successfully applied in other degraded ecosystems (Blumenthal et al., 2003; Eschen et al., 2006; Reever Morgan and Seastedt, 1999) to reduce soil N and alien grass growth (Blumenthal et al., 2003; Eschen et al., 2006), would probably be inappropriate due to the low decomposition rates in most Mediterranean-climate sclerophyllous vegetation (Read and Mitchell, 1983). This premise is supported by the reported inconclusive effects of carbon-rich mulching on soil N levels, alien grass cover and the re-establishment of native plants in transformed lowland fynbos (Holmes, 2008).

The virtual complete depletion of indigenous species in the severely transformed renosterveld old fields and the high mortalities observed among the introduced native species leave only one potentially feasible restoration approach, namely the transfer of seed-bearing soils from adjacent natural vegetation into the transformed areas (Hölzel and Otte, 2003). The advantages associated with such soil transfers are that the regenerating species, unlike those cultivated in nurseries, invest more during their early developmental stages into root than shoot development, which assists their survival over the dry summer period. Also, the entire species complement, including rare species and mutualists, such as mycorrhizae, which are known to assist in plant nutrient uptake (Hölzel and Otte, 2003), are introduced in such soil transfers and the genetic variability of locally adapted ecotypes and races is preserved and maintained (Hölzel and Otte, 2003). Although soil transfers are potentially expensive, the application of local labor in a poverty alleviation scheme could defray these costs by providing employment to local communities and involving them in restoration initiatives (Musil et al., 2005).

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